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## Mechanism of Transcription Activation at the *comG* Promoter by the Competence Transcription Factor ComK of *Bacillus subtilis*

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**The development of genetic competence in *Bacillus subtilis* is regulated by a complex signal transduction cascade, which results in the synthesis of the competence transcription factor, encoded by *comK*. ComK is required for the transcription of the late competence genes that encode the DNA binding and uptake machinery and of genes required for homologous recombination. In vivo and in vitro experiments have shown that ComK is responsible for transcription activation at the *comG* promoter. In this study, we investigated the mechanism of this transcription activation. The intrinsic binding characteristics of RNA polymerase with and without ComK at the *comG* promoter were determined, demonstrating that ComK stabilizes the binding of RNA polymerase to the *comG* promoter. This stabilization probably occurs through interactions with the upstream DNA, since a deletion of the upstream DNA resulted in an almost complete abolishment of stabilization of RNA polymerase binding. Furthermore, a strong requirement for the presence of an extra AT box in addition to the common ComK-binding site was shown. In vitro transcription with *B. subtilis* RNA polymerase reconstituted with wild-type  $\alpha$ -subunits and with C-terminal deletion mutants of the  $\alpha$ -subunits was performed, demonstrating that these deletions do not abolish transcription activation by ComK. This indicates that ComK is not a type I activator. We also show that ComK is not required for open complex formation. A possible mechanism for transcription activation is proposed, implying that the major stimulatory effect of ComK is on binding of RNA polymerase.**

Genetic or natural competence is a physiological differentiation state in which bacteria are able to take up exogenous DNA from the medium. This phenomenon has been best studied in the gram-positive soil bacterium *Bacillus subtilis*. Competence development is initiated at the onset of stationary growth as a result of a complex regulatory cascade. Through quorum sensing, environmental signals such as nutrient availability and cell density are sensed and interpreted (16, 34). Regulation by this cascade leads to the synthesis of the competence transcription factor (CTF), encoded by *comK*. Via an autoregulatory loop, ComK stimulates the expression of its own gene. In addition to this, ComK is required for the transcription of the late competence genes, *comC*, *-E*, *-F*, and *-G*, which encode the DNA binding and uptake machinery and *addAB* and *recA*, which are necessary for DNA recombination and integration (14, 31, 32, 37, 50, 69).

Previously, we have described the mechanism of binding of ComK to the promoter regions of specific competence genes (35, 69). A transcriptional fusion of the *comG* promoter with *lacZ* showed that in vivo expression of *comG* was completely abolished in a *comK* deletion mutant (68). In vitro transcription studies confirmed that ComK alone is capable of activating transcription at the *comG* promoter (35).

Transcriptional regulation by activators has been shown to affect transcription initiation at one or more of the following steps: (i) stimulation of RNA polymerase (RNAP) binding, (ii) stimulation of the isomerization to an open promoter complex, and (iii) helping in promoter clearance (1, 2, 44, 45). The free energy of reaction intermediates of transcription initiation can be limiting at any of these steps. Activator interactions could function in lowering the energy barrier of the rate-limiting step or steps in order to accelerate the overall transcription initiation reaction (27).

The possible interactions at the promoter site that could lead to transcriptional activation are generally divided into three separate levels: (i) direct protein-protein contacts between the activator protein and RNAP; (ii) conformational changes transmitted by the DNA upon binding of the activator; and (iii) additional contacts with other DNA segments than the core promoter sequence, such as interactions between the DNA sequence upstream of the activator binding site and the backside of RNAP (15, 17). It has been postulated that the mechanism of activation depends on the architecture of the promoter as well as on the steps that are rate limiting in transcription initiation for that promoter (38).

In the experiments reported here, we investigated the mechanism of ComK-dependent stimulation of transcription at the *comG* promoter. We demonstrate that RNAP binding to the promoter is stimulated by ComK and that stabilization of binding requires the presence of the upstream region of the promoter DNA. Furthermore, we show that C-terminal deletions in the  $\alpha$ -subunit of RNAP do not abolish transcription activation by ComK. Isomerization to the open complex promoter is shown to be independent of ComK. The implications of these

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant features	Source or reference
<b>Strains</b>		
<i>B. subtilis</i>		
8G5	<i>trpC2 his met tyr-1 ade nic ura</i>	8
NIG2001	<i>trpC2 pheA2</i> Neo <sup>r</sup> <i>rpoC</i> <sub>his6</sub>	25
8GG	<i>amyE::comG-lacZ</i> fusion	This work
8GG6	<i>amyE::comG+6-lacZ</i> fusion	This work
<i>E. coli</i> BL21(DE3)	F <sup>-</sup> <i>ompT hsdS<sub>B</sub></i> (r <sub>B</sub> <sup>-</sup> m <sub>B</sub> <sup>-</sup> ) <i>gal dcm</i>	Novagen
<b>Plasmids</b>		
pCD2	Overexpression of <i>B. subtilis</i> $\sigma^A$	13
pAN-G	Ap <sup>r</sup> , pAN583 with <i>comG</i> promoter	35
pAN-G+6	Ap <sup>r</sup> , mutant <i>comG</i> promoter	This work
pAN-G-AT2-GC	Ap <sup>r</sup> , random high-GC DNA upstream of <i>comG</i> ; deletion of first AT box	This work
pAN-G-AT3-GC	Ap <sup>r</sup> , random high-GC DNA upstream of <i>comG</i>	This work
pAN-G-AT2-sipS	Ap <sup>r</sup> , <i>sipS</i> DNA (high AT) upstream of <i>comG</i> ; deletion of first AT box	This work
pAN-G-AT3-sipS	Ap <sup>r</sup> , <i>sipS</i> DNA (high AT) upstream of <i>comG</i>	This work
pAN-G-AT2-codY	Ap <sup>r</sup> , <i>L. lactis</i> $\Delta$ <i>codY</i> DNA (high AT) upstream of <i>comG</i> ; deletion of first AT box	This work
pAN-G-AT3-codY	Ap <sup>r</sup> , <i>L. lactis</i> $\Delta$ <i>codY</i> DNA (high AT) upstream of <i>comG</i>	This work
pBTK-G	Ap <sup>r</sup> Km <sup>r</sup> , <i>comG-lacZ</i> fusion	This work
pBTK-G+6	Ap <sup>r</sup> Km <sup>r</sup> , <i>comG+6-lacZ</i> fusion	This work

results for the transcription activation mechanism of ComK at the *comG* promoter are discussed.

#### MATERIALS AND METHODS

**Strains, plasmids, and plasmid construction.** The plasmids and bacterial strains used in this study are listed in Table 1.

Plasmid pAN-G+6 was constructed by long-range PCR using primers G+6F and G+6R with pAN-G as the DNA template. Ligation of the subsequent PCR product yielded pAN-G with a 6-bp insertion in between the ComK-binding sites and the promoter -35 sequence. Primers were designed to create a unique *Hind*III restriction site at the place of insertion. Both the wild-type and mutant *comG* promoter fragments were cloned into the pBTK2 *amy*-locus integration vector (46) as a *Bam*HI-*Eco*RI restriction fragment. The resulting plasmids, pBTK-G and pBTK-G+6, were transformed to *B. subtilis* 8G5, and  $\beta$ -galactosidase assays were performed as described previously (67).

The pAN-G-AT-GC plasmids were constructed by PCR with pAN-G as the DNA template and with primers G2trn-*Xho*I and G1trn-*Xho*I (for AT2) or G3trn-*Xho*I (for AT3). The PCR products were digested with *Xho*I and ligated, resulting in plasmids in which the upstream DNA of *comG* is deleted and replaced by high-GC (55%) DNA from the pUC origin. The pAN-G-AT-sipS plasmids were constructed by PCR with primers G2trn-*Xho*I and AT-AT2 (for AT2) or AT-AT3 (for AT3). The PCR products were digested with *Xho*I and *Bpi*I. In this fragment, a PCR product was ligated, made with primers SipS-*Xho*I and SipS-*Bpi*I on chromosomal DNA of *B. subtilis* 8G5 as a template, and digested with *Xho*I and *Bpi*I. In the resulting plasmids, the *comG*-specific upstream DNA is replaced by upstream DNA of *sipS* (61% AT). The plasmid pAN-G-AT-codY was constructed by PCR on pAN-G as a DNA template with primers G2trn-*Xho*I and G1trn-*Xho*I or G3trn-*Xho*I for AT2 and AT3 respectively. Into this fragment, an internal gene fragment of *Lactococcus lactis* *codY* was ligated, made on chromosomal DNA with primers cod20 and cod21. In the resulting plasmids, the upstream DNA of *comG* is replaced by high-AT (60%) DNA of *codY* origin. Use of primer G3trn or AT-AT3 leads to the deletion of one possible AT box in the upstream region of *comG*.

**DNA manipulations and materials.** Standard molecular biology methods (3) were used unless otherwise specified. Enzymes were purchased from Boehringer Mannheim, New England Biolabs, Promega, or Pharmacia. DNA oligonucleotides were synthesized by Gibco BRL or Invitrogen. Radiolabeled nucleotides were obtained from Amersham. The media for growth of *B. subtilis* and *Escherichia coli* have been described previously (3, 70). *B. subtilis* chromosomal DNA was isolated and purified as described previously (70). ComK was purified in this laboratory by the method of Hamoen et al. (35).

**PCR amplifications.** PCRs were carried out as described by Innes and Gelfand (40) by use of *Pwo* DNA polymerase (Boehringer Mannheim GmbH) and *B. subtilis* 8G5 chromosomal DNA, *L. lactis* chromosomal DNA, or plasmid pAN-G

as a template. The primers used in PCRs are listed in Table 2. Probes for use in electrophoretic mobility shift assays were made by PCR. A combination of the primers G1 and G2 was used to create a *comG* promoter fragment. A combination of the primers G2 and G1trn and primers G2 and AT-AT3 results in a truncated *comG* promoter fragment with two or three AT boxes, respectively. Probes with longer upstream DNA sequences were made with primers G7 and G2 for the wild type, retG-1 and G2 for high-GC DNA, and SipS-*Xho*I or cod20 with G2 for high-AT DNA of *sipS* or *codY* origin.

**Purification of  $\sigma^A$ -specific RNA polymerase and of  $\sigma^A$  factor.** To purify RNAP, an overnight culture (5  $\mu$ g of neomycin per  $\mu$ l) of *B. subtilis* NIG2001 (25) was diluted 100 times and grown at 37°C in 2 $\times$  tryptone-yeast medium and harvested at time 0. All subsequent procedures were performed at 4°C. Cells were collected by centrifugation for 10 min at 6,000  $\times$  g and washed with ice-cold buffer A (20 mM Tris-HCl [pH 8.0], 0.2 M NaCl, 1 mM 2-mercaptoethanol, 10% glycerol, 10 mM MgCl<sub>2</sub>). Cells were broken with a French press in buffer A containing 0.5 mM phenylmethylsulfonyl fluoride (PMSF). Cell extracts were obtained by centrifugation for 20 min at 20,000 rpm in a SW50 rotor, after adding an additional 0.5 mM PMSF. The supernatant was diluted up to 10 times in buffer A and applied to a Talon resin column (Clontech) or Ni-nitrilotriacetic acid (Qiagen). The loaded column was washed with buffer A and buffer B (buffer A containing 5 mM imidazol) to remove nonspecifically bound proteins. Bound proteins were eluted by increasing the concentration of imidazol up to 50 mM in buffer A. Protein-containing fractions were diluted in low-salt buffer (20 mM

TABLE 2. Oligonucleotide primers used for PCR

Primer	Sequence
G1 .....	5'-CCG GAA TTC ATG GTG ACC ATG TCT GCT-3'
G2 .....	5'-CGC GGA TCC CTC TCC TTT CAA CGC-3'
G7 .....	5'-TTTTGTGCAGCGTGCCCCGC-3'
retG-1 .....	5'-GATTTTGTGTGATGCTCGTCAG-3'
G+6F .....	5'-CTT TGT TTG ATT ACC TTT TCT TCT TTT TC-3'
G+6R .....	5'-CTT GGG AAA ACG TGA TTT TGT GAG ATG-3'
G1trn- <i>Xho</i> I .....	5'-GATCCTCGAGAGAATTGGTTTTCAGCATATAAC-3'
G2trn- <i>Xho</i> I .....	5'-CTAGCTCGAGGGGTACCGAGCTCGAATTCCG-3'
G3trn- <i>Xho</i> I .....	5'-GATCCTCGAGGAAAGTCTTTTCTTGCCA-3'
AT-AT2 .....	5'-CATGGAAGACTGGGTAAGAATTGGTTTTCAGCA TATAAC-3'
AT-AT3 .....	5'-CATGGAAGACTGGGTAGAAAGTCTTTTCTTGC CAG-3'
cod20 .....	5'-ACACCATGGCTACATTACTTGAAAAACACG-3'
cod21 .....	5'-ATAGAATTCCTCTGACTTTTAGAAATTACGTCG-3'
SipS- <i>Xho</i> I .....	5'-CATGCTCGAGAACTGCCGGAATATATATTGG-3'
SipS- <i>Bpi</i> I .....	5'-CATGGAAGACTCTACCCACATCATGCC-3'

Tris-HCl [pH 8.0], 10 mM MgCl<sub>2</sub>, 20% glycerol, 1 mM 2-mercaptoethanol) and applied to a prepacked disposable 5-ml heparin-agarose column (Pharmacia). After extensive washing with low-salt buffer, RNAP was eluted by increasing the concentration of NaCl in buffer A up to 1.2 M. Finally the sample was dialyzed against cold dialysis buffer (10 mM Tris-HCl [pH 8.0], 7.5% glycerol, 1 mM 2-mercaptoethanol).  $\sigma^A$  was purified from inclusion bodies in *E. coli* as described by Chang and Doi (13). Before use in gel retardation or in vitro transcription reactions, holoenzyme was reconstituted on ice for at least 10 min by mixing RNAP and  $\sigma^A$  in a 1:1 molar ratio.

**Gel retardation analysis.** Gel retardation with ComK and RNAP was carried out essentially as described previously (69). The PCR-generated DNA probes were end labeled with T4 polynucleotide kinase by use of [ $\gamma$ -<sup>32</sup>P]ATP. The purified proteins and probes were premixed on ice in 20  $\mu$ l of binding buffer, consisting of 20 mM Tris-HCl (pH 8.0), 5 mM MgCl<sub>2</sub>, 100 mM KCl, 0.5 mM dithiothreitol (DTT), 0.05-mg/ml poly(dI-dC), 0.05-mg/ml bovine serum albumin (BSA), and 8.7% glycerol. All reactions were performed in the presence of 200  $\mu$ M ATP and 200  $\mu$ M GTP, with the exception of those producing the data shown in Fig. 5, when indicated. Binary complexes were formed by incubation for 15 min at 37°C. To distinguish open RNAP-promoter complexes, 2  $\mu$ l of a 0.3% heparin solution was added directly prior to electrophoresis on a nondenaturing 4% polyacrylamide gel. Gels were run in TAE buffer (40 mM Tris-acetate [pH 8.2], 2 mM EDTA) at 100 V, dried, and autoradiographed.

**In vitro transcription assays.** Reaction mixtures for in vitro transcription analyses contained the following (in 25  $\mu$ l): 25 mM Tris-HCl (pH 7.5); 10 mM MgCl<sub>2</sub>; 100 mM KCl; 1 mM DTT; 45 mM ammonium sulfate; 200  $\mu$ M (each) UTP, ATP, and GTP; 80  $\mu$ M [ $\alpha$ -<sup>32</sup>P]CTP (2  $\mu$ Ci); 1  $\mu$ g of poly(dI-dC); 1  $\mu$ g of BSA; and 9 nM template DNA. The templates used were supercoiled pAN-G and derivatives (34) and a 260-bp DNA fragment containing the phage  $\phi$ 29 C2 promoter (47), which give rise to transcripts of 360 and 98 nucleotides, respectively. Nonreconstituted RNAP and RNAPs reconstituted with deletion mutants of the  $\alpha$ -subunit ( $\Delta$ 15,  $\Delta$ 37, and  $\Delta$ 59) were used as described by Mencia et al. (47). ComK protein was added to a final concentration of 0.35  $\mu$ M. Reactions were performed at 37°C and processed as described previously (51, 54). Transcripts were separated by denaturing polyacrylamide gel electrophoresis and quantified by using a Fuji BAS-III image analyzer.

## RESULTS

### ComK stimulates binding of RNAP at the *comG* promoter.

The basal prokaryotic promoter consists of four critical elements: i.e., the -35 and -10 hexamers, the spacer length between these two hexamers, and upstream auxiliary elements. DNA sequences that resemble the consensus of such a core region are efficient binding sites for RNAP. Nevertheless, they may be poor promoters without the presence of activator proteins (7, 20, 24). The presence of a -35 consensus hexamer for RNAP is important for efficient binding of  $\sigma^A$ -RNAP to the promoter, since  $\sigma^A$  makes specific interactions with DNA at this region (30). In general, the homology score of promoter sequences correlates closely with the in vitro binding affinity of  $\sigma^A$ -RNAP (9, 20, 24). Since the *comG* promoter shows good homology to the  $\sigma^A$  consensus promoter (Fig. 1), the transcription properties of  $\sigma^A$ -RNAP at the *comG* promoter were analyzed by in vitro transcription studies (35). It was shown that in the absence of ComK, hardly any transcripts are formed, while in the presence of ComK, transcription is stimulated up to 50-fold, showing that ComK is sufficient and is required to activate transcription at the *comG* promoter.

Binding properties of  $\sigma^A$ -RNAP at the *comG* promoter were analyzed with electrophoretic mobility shift assays. RNAP was shown to bind to the *comG* promoter also in the absence of ComK. In the presence of ComK, the amount of complexes formed increased two- to fivefold (Fig. 2), resulting in a super-shifted complex. This result suggests that ComK stimulates binding of RNAP to the *comG* promoter.

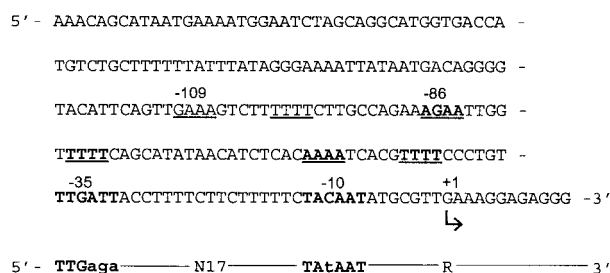


FIG. 1. Nucleotide sequence of the promoter region of the *comG* operon from bp -204 to +12. The nucleotides are numbered corresponding to the in vivo transcriptional start site from the *comG* promoter. In the *comG* promoter, three potential ComK dimer binding sites (AT boxes) are present (underlined). The site indicated in boldface and underlined is located at a position comparable to the ComK-binding site in other ComK-regulated promoters. The position of the starting nucleotide (italic) of the common AT box (position -86) is indicated, as well as the starting position of the extra AT box (position -109). The proposed -10 and -35 promoter sequences are printed in boldface. The *B. subtilis*  $\sigma^A$  consensus promoter is depicted underneath the *comG* sequence for comparison (bases in capital letters are present in >70% of the promoters) (36).

**RNAP binding is stabilized by ComK through the upstream DNA region.** When a truncated *comG* promoter fragment, lacking the DNA upstream of the ComK-binding sites, was used in an electrophoretic mobility shift assay under the same conditions, the stabilization of the RNAP-promoter complex was abolished (Fig. 3A), although binding of ComK or RNAP alone was not disturbed. Therefore, it can be concluded that for stabilization of the complex, the DNA upstream of the ComK boxes is important.

Stimulatory effects of upstream DNA on transcription activation are known for several promoters (17, 61, 62). Often, a specific activation sequence, the UP element, can be distinguished, consisting of an AT-rich region located between -40 and -60 relative to the transcription start site (22, 23, 29). In the case of the *comG* promoter, this region is occupied by ComK binding, but the DNA-bending capacities of ComK suggest a possible specific sequence to be located further upstream of the promoter (59). To test whether the importance of the upstream DNA in the case of stable RNAP binding at the *comG* promoter is a result of the presence of a specific sequence or of the structural presence of DNA, mutants were constructed in which the upstream DNA of *comG* was replaced by nonspecific DNA, with either a high GC content or a high AT content. Furthermore, two types of constructs were tested, which differed in the number of possible ComK-binding sites upstream of the promoter. Commonly, one K box, consisting of two AT boxes is present upstream of a ComK-activated gene (35). In the case of *comG*, an extra AT box is located one helical turn upstream of the common K box. In one set of mutants, called AT3, all three AT boxes were present, while in the other set of mutants, called AT2, only two boxes were present (Fig. 3C).

The different promoters were tested by electrophoretic mobility shift assays and in vitro transcription assays (Fig. 3B), showing that deletion of the third AT box resulted in an almost complete loss of stabilization of RNAP binding and transcription in the presence of ComK. This result indicates that the presence of the third AT box is of great importance for tran-



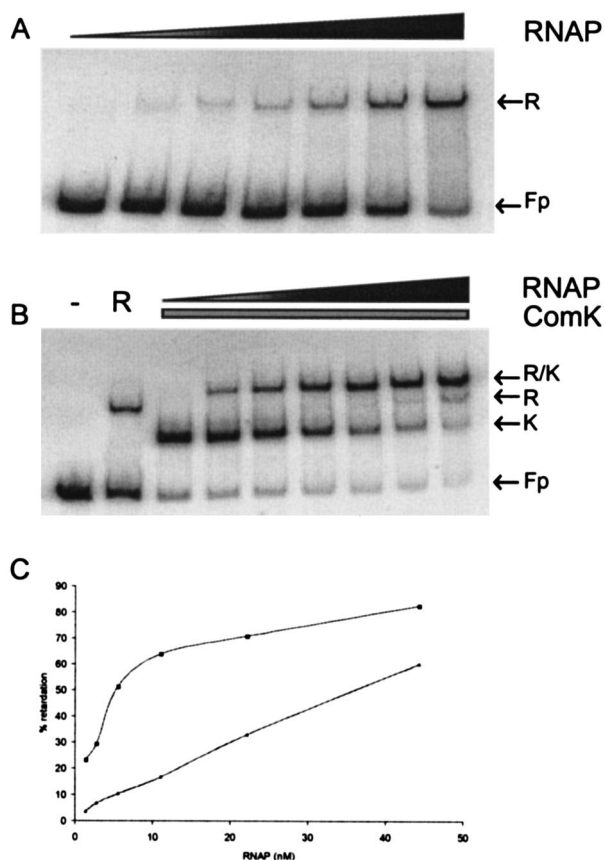


FIG. 2. Complexes formed by RNAP and the *comG* promoter with or without ComK present. Electrophoretic mobility shift assays were performed with a 200-bp  $^{32}$ P-labeled *comG* promoter fragment. RNAP concentrations were increased in twofold increments from 0 to 44 nM, as indicated by the concentration bars. The positions of the different complexes are shown on the right. K, ComK; R, RNAP; Fp, free probe. (A) RNAP binding in the absence of ComK. (B) RNAP binding in the presence of 0.35  $\mu$ M ComK. For comparison, a blank sample (-) and binding of only RNAP (R; 22 nM) are shown in the first and second lanes, respectively. (C) The percentage of radioactive probe in the RNAP-promoter complex as determined by densitometric scanning was plotted against nanomolar RNAP concentration. The percentage was calculated by dividing the signal of the RNAP band by the total signal in each lane, which was determined by combining the intensities of the bands present per lane. Triangles, no ComK present; squares, 0.35  $\mu$ M ComK.

scription activation at the *comG* promoter. However, the box alone is not sufficient to stabilize RNAP binding, since stabilization is still almost completely abolished when a truncated *comG* promoter lacking the DNA upstream of the three AT boxes is used. The replacement of the *comG*-specific upstream DNA with either high-GC or high-AT DNA showed only a slight reduction of stabilization of RNAP binding, which never exceeded a 2- to 2.5-fold difference.

**ComK is not a type I transcriptional activator.** An important class of prokaryotic transcription factors mediates transcription activation through direct contacts with the RNAP. A preferred activation target is the C-terminal domain of the  $\alpha$ -subunit of RNAP (6, 21). In general, those activators binding at or upstream from position -60 relative to the transcription start site normally interact with the  $\alpha$ -subunit (41). To inves-

tigate whether ComK stimulates transcription through contacts with the  $\alpha$ -subunit, in vitro transcription assays were performed using RNAPs reconstituted with either wild-type  $\alpha$ -subunit or  $\alpha$ -subunits lacking the last 15, 37, or 59 amino acids from the carboxyl-end, respectively. Equivalent amounts of the reconstituted RNAPs were added to the transcription reaction mixtures, and the reaction products were separated by electrophoresis. The results demonstrated that the RNAPs containing deletion mutants of the  $\alpha$ -subunit were still stimulated by ComK (Fig. 4A), suggesting that direct protein-protein contacts between ComK and the  $\alpha$ -subunits are not re-

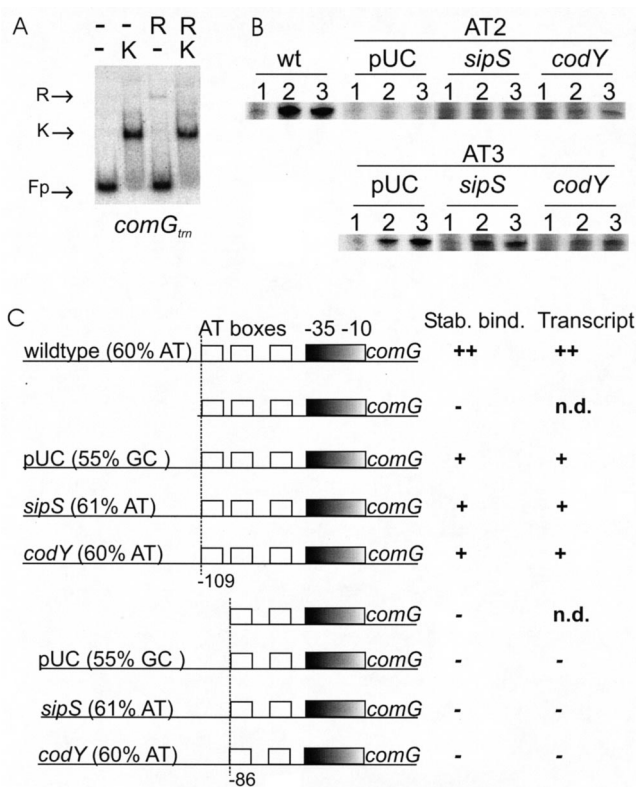


FIG. 3. The upstream region of the *comG* promoter is responsible for stabilizing the RNAP-promoter complex. (A) Electrophoretic mobility shift experiments were performed with a  $^{32}$ P-labeled truncated *comG* (*comG*<sub>tm</sub>, two AT boxes, no upstream DNA) promoter fragment. Reaction mixtures contained RNAP (17.5 nM) and/or ComK (0.35  $\mu$ M), as indicated above the lanes. The positions of the different complexes are shown on the right. K, ComK; R, RNAP; Fp, free probe. (B) In vitro transcription reactions were performed as described in Materials and Methods on pAN-G or derivatives containing wild-type (wt) upstream DNA or nonspecific upstream DNA, as indicated in panel C. AT2, two AT boxes; AT3, three AT boxes; 1, no ComK present; 2, 0.07  $\mu$ M ComK; 3, 0.35  $\mu$ M ComK. (C) Schematic overview of the constructs tested for the influence of the upstream DNA on stabilization of RNAP binding (Stab. bind.) and transcription (Transcript). n.d., not determined. The AT boxes are represented as squares, and the RNAP-binding site is represented as a rectangle. The fragments contain upstream DNA (300 bp) of wild-type *comG* origin, high-GC DNA, high-AT DNA, or no upstream DNA. The dotted lines indicate at which position of the wild-type *comG* promoter the fusions or truncations were made. (The numbers correspond to those in Fig. 1.) The situations with nonspecific upstream DNA or no upstream DNA were tested for both three and two AT boxes. ++, wild-type stabilization of RNAP binding or transcription in the presence of ComK (100%); +, 40 to 80% stabilization; -, 0 to 10% stabilization.

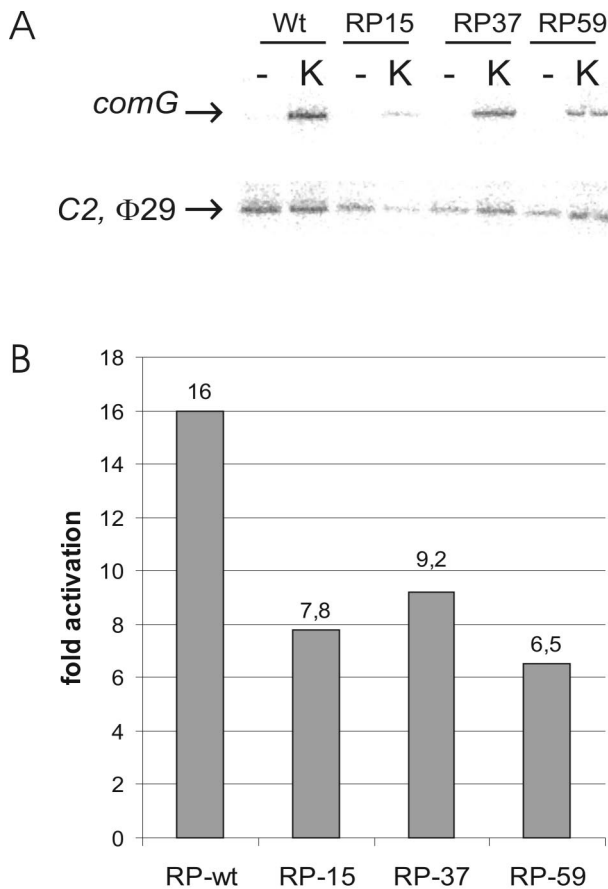


FIG. 4. ComK stimulation of transcription by RNAP containing wild-type (Wt) or mutant  $\alpha$ -subunits. (A) In vitro transcription reactions were performed as described in Materials and Methods. Products were separated by electrophoresis. The constitutive C2 promoter from phage  $\Phi$ 29 was used as an internal standard for specific activity of reconstituted RNAPs. (B) Transcription products were quantified with a Fuji BAS-III image analyzer. Transcription is reported as fold stimulation over that with no ComK added for each RNAP preparation, corrected for differences in specific RNAP activity by use of the internal standard. The abbreviations RP-wt, RP-15, RP-37, and RP-59 represent RNAP reconstituted with the wild-type  $\alpha$ -subunit and the  $\Delta$ 15,  $\Delta$ 37, and  $\Delta$ 59 deletion mutants, respectively.

quired for transcription activation. However, the maximum level of transcription by RNAP reconstituted with the mutant  $\alpha$ -subunits was reduced approximately twofold compared to that of the wild-type polymerase (Fig. 4B). This suggests that the C-terminal domain of the  $\alpha$ -subunit is important for optimal transcription activity, as will be discussed.

Using electrophoretic mobility shift assays, it was also shown that ComK did not promote the binding of purified  $\alpha$ -subunits (wild type and deletion mutants) nor that of purified  $\sigma^A$  to the promoter (results not shown). Thus, it can be concluded that direct protein-protein contacts between ComK and the  $\alpha$ - or  $\sigma$ -subunits of RNAP are not required for stabilizing RNAP binding to the promoter.

**Open complex formation at the *comG* promoter is independent of ComK.** The second step in transcription initiation is open complex formation. Competitor resistance is widely used as a functional assay for open complex formation (66, 71). Heparin challenge experiments indicated that the presence of

ComK is not required for formation of open complexes at the *comG* promoter (Fig. 5A). Open complex formation was shown to be dependent on the presence of the initiating nucleotides. Only when ATP and GTP were added to the reaction mixtures did RNAP-promoter complexes become resistant to a heparin challenge. Upon addition of both nucleotides, an additional stabilizing effect on RNAP binding and an additional shift were observed, compared with the complexes formed in the absence or presence of only one of the nucleotides. This could be caused by the fact that in the presence of both initiating nucleotides a short abortive transcript can be formed (66). Likely, the formation of a short transcript stabilizes the binding of RNAP and causes a slightly altered migration pattern upon electrophoresis.

Normal open complex formation was also seen when the truncated *comG* promoter fragment with two AT boxes was used in the heparin challenge experiments (results not shown). These results confirm that when RNAP is bound to the promoter, open complex formation occurs upon the addition of nucleotides and independent of ComK.

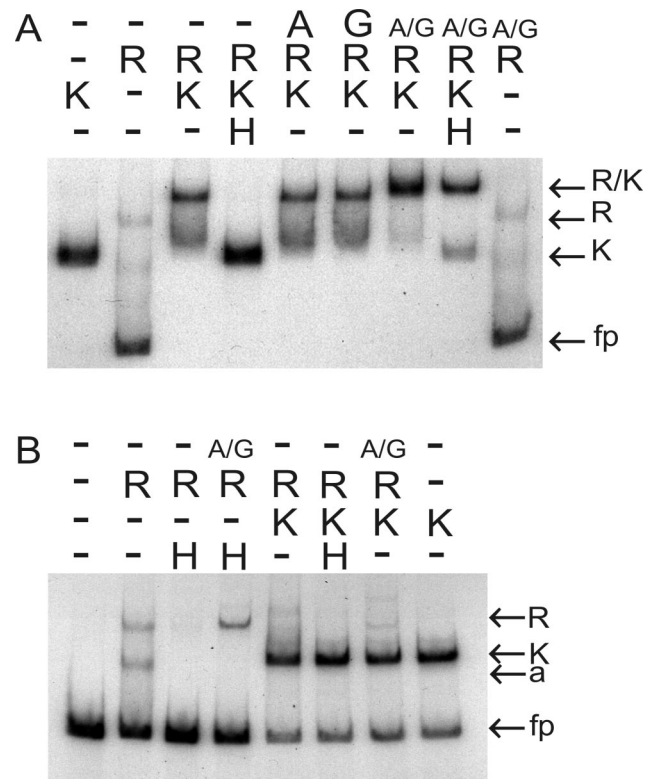


FIG. 5. (A) Requirement of initiating nucleotides for heparin resistance. Electrophoretic mobility shift experiments were performed with the  $^{32}$ P-labeled *comG* promoter fragment. (B) Helix face dependency of the ComK effect on RNAP binding to the promoter. Electrophoretic mobility shift experiments were performed with the  $^{32}$ P-labeled *comG*+6 (206-bp) promoter fragment. For both panels A and B, reaction mixtures contained RNAP (17.5 nM), ComK (0.35  $\mu$ M), and initiating nucleotides (200  $\mu$ M) as indicated above the lanes. Heparin challenge was performed as described in Materials and Methods. The positions of the different complexes are shown on the right. K, ComK; R, RNAP; H, heparin; A, ATP; G, GTP; fp, free probe; a, aspecific binding in lane 2, connected with the purified RNAP sample.

**Transcription activation is helix face dependent.** To investigate whether the orientation of the ComK-binding sites is important for transcription activation, a mutant *comG* promoter fragment was created that contained a 6-bp insertion between the ComK box and the  $-35$  hexamer. In this situation, the ComK-binding site is located on the opposite face of the DNA helix, and as a consequence, the bound ComK dimers are on the opposite face of the helix compared to the downstream RNAP. It has been found that ComK induces a bend in the DNA upon binding (35). In the *comG*+6 construct, this ComK-induced bend is present in the opposite direction compared to the wild-type situation.

The *comG*+6 promoter fragment was used in in vitro transcription assays, which showed abolishment of activation of transcription by ComK. In vivo, this promoter was placed in front of the *lacZ* gene in the *amy* locus of the *B. subtilis* chromosome.  $\beta$ -Galactosidase expression was abolished to the same level as in a *comK* deletion mutant (68; results not shown).

Electrophoretic mobility shift assays with *comG*+6 showed that stabilization of RNAP binding was disturbed, a situation comparable to that with the truncated *comG* promoters. Still, the initial level of RNAP binding in the absence of ComK was the same as that in the wild-type *comG* promoter, as was open complex formation (Fig. 5B). Binding of RNAP seemed to be lost when ComK bound to the other side of the helix. These results indicate a strict helix face dependency for transcription activation by ComK. It has been proposed that intrinsic or protein-induced DNA bends immediately upstream of a promoter site can activate transcription by looping the upstream DNA sequences around to interact with the backside of RNAP (56, 58, 59). This would explain both the requirement for the upstream region of the *comG* promoter and the helix face dependency.

## DISCUSSION

Transcription initiation frequently requires the interaction of several DNA binding proteins that ultimately modulate the activity of RNAP. In competence development in *B. subtilis*, *comK* encodes the central regulator, also known as the CTF. ComK activates and binds specifically to the promoters of the late competence genes and the genes required for recombination. In vitro studies have shown that purified ComK alone is capable of activating transcription at the *comG* promoter. In this report, we describe the mechanism of this transcriptional activation.

In order to see in which step of transcription initiation ComK is involved, several approaches were taken. Using electrophoretic mobility shift assays, it was shown that RNAP can bind to the *comG* promoter also in the absence of ComK, but that the amount of closed complexes is stimulated up to fivefold when ComK is present (Fig. 2).

Stabilization of the RNAP-promoter complex in the presence of ComK was shown to be dependent on the DNA upstream of the ComK-binding sites. When this upstream DNA was deleted, binding of ComK and RNAP alone to the fragment was not disturbed (Fig. 3A), but the supershifted RNAP-promoter complex was no longer stabilized, suggesting that stabilization of RNAP binding is a result of bending of the

upstream DNA by ComK, thereby enabling interactions between the DNA and RNAP.

Replacement of the *comG*-specific upstream DNA by either high-GC or high-AT DNA resulted in only a slight reduction in stabilization of RNAP binding and transcription (Fig. 3C). Several sequence comparisons were made between the upstream DNA of *comG* and those of other ComK-activated genes. No clear conserved sequences could be indicated, but a major difference between *comG* and other ComK-activated genes is the presence of an extra AT box upstream of *comG*. Binding assays and in vitro transcription studies comparing promoter fragments with either two or three AT boxes upstream of the *comG* gene showed the requirement of the third box for stabilization of RNAP binding and transcription in vitro (Fig. 3C). Previous footprinting studies by Hamoen et al. demonstrated that all three AT boxes are protected by ComK (35). The presence of this extra AT box might be the determinant that results in the large transcription at the *comG* promoter. Array studies indicated that *comG* transcription is the highest of all ComK-activated genes, and in vitro transcription studies with ComK-activated genes have thus far only been successful for *comG* (4, 33, 55). Studies with a truncated *comG* promoter that still contained all three AT boxes but lacked the upstream DNA no longer showed stabilization of RNAP binding, indicating that, in addition to the third AT box, the presence of more upstream DNA is required.

The requirement for upstream DNA correlates with the results shown in in vitro transcription assays with RNAPs reconstituted with the wild type or C-terminal deletion mutants of the  $\alpha$ -subunit. The results indicated that a direct interaction between the  $\alpha$ -C-terminal domain (CTD) and ComK is not required for RNAP activation (Fig. 4), since ComK could still stimulate transcription by mutant RNAPs. Electrophoretic mobility shift assays showed that ComK is not able to recruit purified  $\alpha$ -subunit or  $\sigma^A$  to the *comG* promoter, another indication that no significant contacts between ComK and RNAP are involved in activation.

Although transcription activation was not abolished in the reconstituted mutant RNAPs, a twofold reduction in maximal transcription was observed. Rowe-Magnus et al. (63) reported a similar observation for the transcription of the *spoIIIG* promoter by Spo0A~P. They suggested an effect on the interaction of RNAP with promoter DNA by the  $\alpha$ -subunit mutation. The CTD of the  $\alpha$ -subunit is known to interact with additional promoter sequences (UP elements) to stabilize polymerase-DNA interactions at some promoters (17, 22, 23, 29, 61, 62). Although in the upstream region of *comG*, a clear UP element could not be demonstrated, it still is possible that specific AT-rich stretches in the upstream DNA interact with RNAP. In *E. coli*, the same residues of the  $\alpha$ -CTD were found to be involved in interaction with activators such as CRP and promoter UP elements (52). If the  $\alpha$ -subunit CTD would indeed help to stabilize the binding of RNAP to the promoter, it would explain why deletions in this domain disturb optimal transcription activity and why the presence of upstream DNA is important for optimal RNAP binding.

Although we cannot totally rule out the possibility that ComK interacts with RNAP through some other region of the enzyme than the  $\alpha$ -subunits or  $\sigma^A$ , like the  $\beta$ - or  $\beta'$ -subunit, we currently favor the notion that ComK activation of transcription



from the *comG* promoter is mediated via stabilization of RNAP binding through the upstream region of the promoter DNA.

Each step in the transcription initiation process is in principle a target for regulation by transcriptional activators (27). Activation can involve multiple interactions between a single activator molecule and the transcription machinery, each interaction being responsible for a specific mechanistic consequence. In fact, such multiple interactions have become a commonly observed feature in transcription activation (11, 38, 53). To advance our understanding of the effect of ComK on the transcription initiation process at the *comG* promoter, several experiments were performed to investigate in which step of transcription initiation ComK is involved.

When we inverted the orientation of the ComK- and RNAP-binding sites in the *comG*+6 promoter construct, stabilization of RNAP binding to the promoter was abolished. In the presence of ComK, no basal level of RNAP binding was observed, suggesting that binding of ComK to the opposite face of the helix hinders RNAP binding to the promoter. In addition, in vitro and in vivo transcription from this promoter was lost. Helix face dependency has been taken as evidence for cross-talk between RNAP and the activator protein (28). Since protein-protein interactions with the  $\alpha$ - or  $\sigma$ -subunits of RNAP are not involved in transcription activation by ComK, it is likely that the orientation of the DNA bend caused by ComK binding is responsible for the helix face dependency. It has been proposed that activator-induced bending of the DNA upstream of the promoter facilitates caging of RNAP to optimize the promoter (2, 10, 59). We conclude that the mechanism of activation relies on contacts between the DNA upstream of the ComK-binding sites and the backside of RNAP. Similar findings have been reported for the *gal* and *lac* promoters (15) and for the CRP-dependent *malT* promoter (18).

For the in vitro transcription assays, supercoiled templates were used, because we found them to be approximately 20-fold more efficient in transcription than runoff transcription assays using the linear template (results not shown). The supercoiled state of the chromosome is known to affect the activity of many promoters (57). It is a fairly common phenomenon among prokaryotic promoters to be stimulated by DNA superhelicity (5, 60). The stimulatory effect of superhelicity of the template on transcription efficiency is also in agreement with our model. The influence of DNA bending on regulatory processes may be modulated by DNA superhelicity (26). Specifically, supercoiling and bending may synergistically enhance polymerase contacts by creating a defined DNA topology at the promoter site, a view also put forward by Zinkel and Crothers (72). Alternatively, DNA supercoiling may optimize the three-dimensional geometry of the DNA for correct alignment of the proteins and/or DNA sites, thus lowering energy barriers in transcription initiation (43).

The *comG* promoter has a strong resemblance to the *B. subtilis* consensus promoter sequence for  $\sigma^A$ -dependent promoters (Fig. 1). In general, such consensus-like promoters stably bind RNAP and require alterations to accelerate the late steps of the transcription initiation pathway (19, 39). Therefore, ComK might also influence transcription initiation in one of the later stages after closed complex formation. The stabilization of the closed complex by ComK will, of course, contribute to accelerate the overall transcription process.

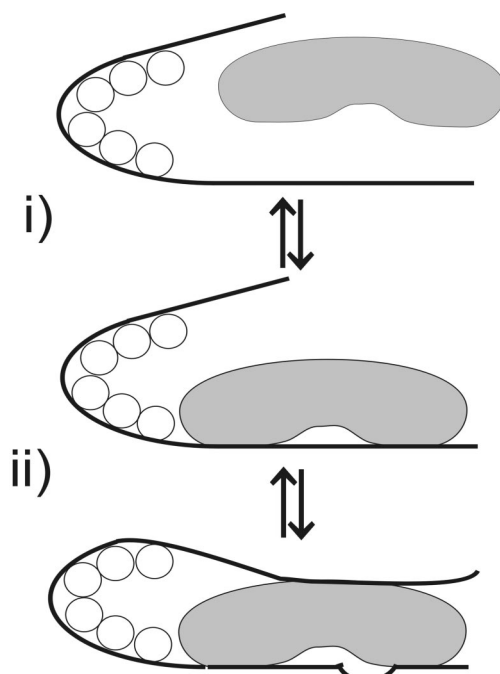


FIG. 6. Model of transcription activation by ComK at the *comG* promoter. ComK proteins are represented as the small circles, RNAP is represented as a large ellipsoid, and DNA is represented by a solid line. For details, see the text.

The second step in transcription initiation, open complex formation, was found to be independent of ComK. Addition of initiating nucleotides was sufficient to induce a heparin-resistant promoter complex. The formation of an open promoter complex is not disturbed when half a helical turn is inserted between the promoter and the ComK boxes. All of this clearly indicates that open complex formation is not a rate-limiting step for transcription initiation at the *comG* promoter.

It has been suggested that consensus  $\sigma^A$  promoters that efficiently bind RNAP and that exhibit strong open complex formation may be limited in the subsequent movement of the polymerase to the elongating complex (12, 39). RNAP binding at these promoters generates a nucleoprotein complex that is too stable to allow promoter clearance (39, 51). Melting of the DNA strands in the promoter region in the presence of NTPs leads to an initiating complex that is trapped in short abortive transcript synthesis (48). The escape from this complex into an elongating transcription machinery involves major conformational changes, including loss of the promoter-specific contacts and the release of the  $\sigma$  factor (42, 49, 65). Escape from abortive initiation has been found to be rate limiting at several other prokaryotic promoters (48, 64). In the case of the *comG* promoter, initial experiments were performed to elucidate the role of ComK in promoter escape of RNAP. To distinguish between an effect of ComK on RNAP binding or promoter escape, ComK had to be added after the binding step. In this case, involvement of ComK in promoter clearance could not be demonstrated, since transcription levels were severely decreased when ComK was added in a later stage of the initiation process than in the binding step. Further research will be required to investigate whether bending of the upstream DNA



around ComK results not only in stabilization of RNAP binding, but also in creating optimal conditions for later steps in the transcription initiation process, like promoter escape.

The proposed model for the role in transcription activation by ComK is summarized in Fig. 6. Although RNAP is capable of binding to the *comG* promoter in the absence of ComK, binding is stimulated when ComK is present (step 1). In the case of the *comG* promoter, ComK can bind to three AT boxes, resulting in bending of the upstream DNA around ComK. This DNA probably interacts with the RNAP, thus stabilizing the RNAP-promoter complex (step 2). Further studies should be performed to see whether interactions between the upstream DNA and the backside of RNAP also help to induce conformational changes in the promoter DNA and/or RNAP that are required for promoter clearance.

In this study, we have investigated the mechanism of transcription activation at the *comG* promoter, which differs from most other ComK-activated genes by containing a third AT box. However, we suggest that the transcription activation mechanism at promoters containing only two AT boxes is comparable to the model presented in this study. It is likely that the major effect of ComK is stabilization of RNAP binding via the upstream DNA region. We suggest that the function of the third AT box is mainly enhancing transcription levels at the *comG* promoter. This view is supported by the fact that the level of expression of the *comG* operon is the highest of all ComK-activated genes.

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#### REFERENCES

- Adhya, S., and S. Garges. 1990. Positive control. *J. Biol. Chem.* **265**:10797–10800.
- Adhya, S., M. Gottesman, S. Garges, and A. Oppenheim. 1993. Promoter resurrection by activators—a minireview. *Gene* **132**:1–6.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.). 1995. Current protocols in molecular biology. Green Publishing Associates and Wiley Interscience, New York, N.Y.
- Berka, R. M., J. Hahn, M. Albano, I. Draskovic, M. Persuh, X. Cui, A. Sioma, W. Widner, and D. Dubnau. 2002. Microarray analysis of the *Bacillus subtilis* K-state: genome-wide expression changes dependent on ComK. *Mol. Microbiol.* **43**:1331–1345.
- Borowiec, J. A., and J. D. Gralla. 1987. All three elements of the *lac*  $p^S$  promoter mediate its transcriptional response to DNA supercoiling. *J. Mol. Biol.* **195**:89–97.
- Boucher, P. E., and S. Stibitz. 1995. Synergistic binding of RNA polymerase and BvgA phosphate to the pertussis toxin promoter of *Bordetella pertussis*. *J. Bacteriol.* **177**:6486–6791.
- Bracco, L., D. Kotlarz, A. Kolb, S. Diekmann, and H. Buc. 1989. Synthetic curved DNA sequences can act as transcriptional activators in *Escherichia coli*. *EMBO J.* **8**:4289–4296.
- Bron, S., and G. Venema. 1972. Ultraviolet inactivation and excision repair in *Bacillus subtilis*. I. Construction and characterization of a transformable eightfold auxotrophic strain and two ultraviolet-sensitive derivatives. *Mutat. Res.* **15**:1–10.
- Brunner, M., and H. Bujard. 1987. Promoter recognition and promoter strength in the *Escherichia coli* system. *EMBO J.* **6**:3139–3144.
- Buc, H. 1986. Mechanism of activation of transcription by the complex formed between cyclic-AMP and its receptor in *Escherichia coli*. *Biochem. Soc. Trans.* **14**:196–199.
- Busby, S., and R. H. Ebright. 1997. Transcription activation at class II CAP-dependent promoters. *Mol. Microbiol.* **23**:853–859.
- Carpousis, A. J., and J. D. Gralla. 1985. Interaction of RNA polymerase with *lacUV5* promoter DNA during mRNA initiation and elongation. *J. Mol. Biol.* **183**:165–177.
- Chang, B.-Y., and R. H. Doi. 1990. Overproduction, purification, and characterization of *Bacillus subtilis* RNA polymerase  $\sigma^A$  factor. *J. Bacteriol.* **172**:3257–3263.
- Chen, I., and D. Dubnau. 2003. DNA transport during transformation. *Front. Biosci.* **8**:544–556.
- Déthiollaz, S., P. Eichenberger, and J. Geiselmann. 1996. Influence of DNA geometry on transcriptional activation in *Escherichia coli*. *EMBO J.* **15**:5449–5458.
- Dubnau, D. 1993. Genetic exchange and homologous recombination, p. 555–584. In A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
- Eichenberger, P., S. Déthiollaz, N. Fujita, A. Ishihama, and J. Geiselmann. 1996. Influence of the location of the cAMP receptor protein binding site on the geometry of transcriptional activation complex in *Escherichia coli*. *Biochemistry* **35**:15302–15312.
- Eichenberger, P., S. Déthiollaz, H. Buc, and J. Geiselmann. 1997. Structural kinetics of transcription activation at the *malT* promoter of *Escherichia coli* by UV laser footprinting. *Proc. Natl. Acad. Sci. USA* **94**:9022–9027.
- Ellinger, T., D. Behnke, H. Bujard, and J. D. Gralla. 1994. Stalling of *Escherichia coli* RNA polymerase in the +6 to +12 region *in vivo* is associated with tight binding to consensus promoter elements. *J. Mol. Biol.* **239**:455–465.
- Ellinger, T., D. Behnke, R. Knaus, H. Bujard, and J. D. Gralla. 1994. Context-dependent effects of upstream A-tracts: stimulation or inhibition of *Escherichia coli* promoter function. *J. Mol. Biol.* **239**:466–475.
- Engelhorn, M., and J. Geiselmann. 1998. Maximal transcriptional activation by the IHF protein of *Escherichia coli* depends on optimal DNA bending by the activator. *Mol. Microbiol.* **30**:431–441.
- Estrem, S. T., T. Gaal, W. Ross, and R. L. Gourse. 1998. Identification of an UP element consensus sequence for bacterial promoters. *Proc. Natl. Acad. Sci. USA* **95**:9761–9766.
- Estrem, S. T., W. Ross, T. Gaal, Z. W. S. Chen, W. Niu, R. H. Ebright, and R. L. Gourse. 1999. Bacterial promoter architecture: subunit structure of UP elements and interactions with the carboxy-terminal domain of the RNA polymerase  $\alpha$  subunit. *Genes Dev.* **13**:2134–2147.
- Frederick, K., T. Caramori, Y. Chen, A. Galizzi, and J. D. Helmann. 1995. Promoter architecture in the flagellar regulon of *Bacillus subtilis*: high-level expression of flagellin by the  $\sigma^D$  RNA polymerase requires an upstream promoter element. *Proc. Natl. Acad. Sci. USA* **92**:2582–2586.
- Fujita, M., and Y. Sadaie. 1998. Rapid isolation of RNA polymerase from sporulating cells of *Bacillus subtilis*. *Gene* **221**:185–190.
- Gartenberg, M. R., and D. M. Crothers. 1991. Synthetic DNA bending sequences increase the rate of *in vitro* transcription initiation at the *Escherichia coli lac* promoter. *J. Mol. Biol.* **219**:217–230.
- Geiselmann, J. 1997. The role of DNA conformation in transcriptional initiation and activation in *Escherichia coli*. *J. Biol. Chem.* **272**:599–607.
- Giladi, H., S. Koby, M. E. Gottesman, and A. B. Oppenheim. 1992. Supercoiling, integration host factor, and a dual promoter system participate in the control of the bacteriophage  $\lambda$   $p_L$  promoter. *J. Mol. Biol.* **224**:937–948.
- Gourse, R. L., W. Ross, and T. Gaal. 2000. UPs and downs in bacterial transcription initiation: the role of the alpha subunit of RNA polymerase in promoter recognition. *Mol. Microbiol.* **37**:687–695.
- Gross, C. A., M. Lonetto, and R. Losick. 1992. Bacterial sigma factors, p. 129–176. In K. Yamamoto and S. McKnight (ed.), *Transcriptional regulation*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Haijema, B. J., L. W. Hamoen, J. Kooistra, G. Venema, and D. Dubnau. 1995. Expression of the ATP-dependent deoxyribonuclease of *Bacillus subtilis* is under competence-mediated control. *Mol. Microbiol.* **15**:203–211.
- Haijema, B. J., D. Van Sinderen, K. Winterling, J. Kooistra, G. Venema, and L. W. Hamoen. 1996. Regulated expression of the *dinR* and *recA* genes during competence development and SOS induction in *Bacillus subtilis*. *Mol. Microbiol.* **22**:75–85.
- Hamoen, L. W., W. K. Smits, A. de Jong, S. Holsappel, and O. P. Kuipers. 2002. Improving the predictive value of the competence transcription factor (ComK) binding site in *Bacillus subtilis* using a genomic approach. *Nucleic Acids Res.* **30**:5517–5528.
- Hamoen, L. W., G. Venema, and O. P. Kuipers. 2003. Controlling competence in *Bacillus subtilis*: shared use of regulators. *Microbiology* **149**:9–17.
- Hamoen, L. W., A. F. van Werkhoven, J. J. E. Bijlsma, D. Dubnau, and G. Venema. 1998. The competence transcription factor of *Bacillus subtilis* recognizes short A/T-rich sequences arranged in a unique flexible pattern along the DNA helix. *Genes Dev.* **12**:1539–1550.
- Helmann, J. D. 1995. Compilation and analysis of *Bacillus subtilis*  $\sigma^A$ -dependent promoter sequences: evidence for extended contact between RNA polymerase and upstream promoter DNA. *Nucleic Acids Res.* **23**:2351–2360.
- Ho, T. T., P. Tortosa, M. Albano, and D. Dubnau. 2002. Rok (YkuW) regulates genetic competence in *Bacillus subtilis* by directly repressing comK. *Mol. Microbiol.* **43**:15–26.
- Hochschild, A., and S. L. Dove. 1998. Protein-protein contacts that activate and repress prokaryotic transcription. *Cell* **92**:597–600.

39. Hsu, L. M. 2002. Promoter clearance and escape in prokaryotes. *Biochim. Biophys. Acta* **1577**:191–207.
40. Innes, M. A., and D. H. Gelfand. 1990. Optimization of PCRs, p. 3–12. *In* M. A. Innes, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, Calif.
41. Ishihama, A. 1993. Protein-protein communication within the transcription apparatus. *J. Bacteriol.* **175**:2483–2489.
42. Knaus, R., and H. Bujard. 1988. P<sub>L</sub> of coliphage lambda: an alternative solution for an efficient promoter. *EMBO J.* **7**:2919–2923.
43. Lewis, D. E. A., M. Geanakopoulou, and S. Adhya. 1999. Role of HU and DNA supercoiling in transcription repression: specialized nucleoprotein repression complex at *gal* promoters in *Escherichia coli*. *Mol. Microbiol.* **31**:451–461.
44. Lloyd, G., P. Landini, and S. Busby. 2001. Activation and repression of transcription initiation in bacteria. *Essays Biochem.* **37**:17–31.
45. McClure, W. R. 1985. Mechanism and control of transcription initiation in prokaryotes. *Annu. Rev. Biochem.* **54**:171–204.
46. Meijer, W. J. J. 1995. Replication and maintenance of plasmids in *Bacillus subtilis*. Ph.D. thesis. University of Groningen, Haren, The Netherlands.
47. Mencia, M., M. Monsalve, F. Rojo, and M. Salas. 1996. Transcription activation by phage  $\phi$ 29 protein p4 is mediated by interaction with the  $\alpha$  subunit of *Bacillus subtilis* RNA polymerase. *Proc. Natl. Acad. Sci. USA* **93**:6616–6620.
48. Menendez, M., A. Kolb, and H. Buc. 1987. A new target for CRP action at the *malT* promoter. *EMBO J.* **6**:4227–4234.
49. Mishra, R. K., and D. Chatterji. 1993. Mechanism of initiation of transcription by *Escherichia coli* RNA polymerase on supercoiled template. *Mol. Microbiol.* **8**:507–515.
50. Mohan, S., and D. Dubnau. 1990. Transcriptional regulation of *comC*: evidence for a competence-specific transcription factor in *Bacillus subtilis*. *J. Bacteriol.* **172**:4064–4071.
51. Monsalve, M., M. Mencia, F. Rojo, and M. Salas. 1996. Activation and repression of transcription at two different phage  $\phi$ 29 promoters are mediated by interaction of the same residues of regulatory protein p4 with RNA polymerase. *EMBO J.* **15**:383–391.
52. Murakami, K., N. Fujita, and A. Ishihama. 1996. Transcription factor recognition surface on the RNA polymerase  $\alpha$  subunit is involved in contact with the DNA enhancer element. *EMBO J.* **15**:4358–4367.
53. Niu, W., Y. Kim, G. Tau, T. Heyduk, and R. H. Ebright. 1996. Transcription activation at class II CAP-dependent promoters: two interactions between CAP and RNA polymerase. *Cell* **87**:1123–1134.
54. Nuez, B., F. Rojo, and M. Salas. 1992. Phage  $\phi$ 29 regulatory protein p4 stabilizes the binding of RNA polymerase to the late promoter in a process involving direct protein-protein contacts. *Proc. Natl. Acad. Sci. USA* **89**:11401–11405.
55. Ogura, M., H. Yamaguchi, K. Kobayashi, N. Ogasawara, Y. Fujita, and T. Tanaka. 2002. Whole-genome analysis of genes regulated by the *Bacillus subtilis* competence transcription factor ComK. *J. Bacteriol.* **184**:2344–2351.
56. Parekh, B. S., and G. W. Hatfield. 1996. Transcriptional activation by protein-induced bending: evidence for a DNA structural transmission model. *Proc. Natl. Acad. Sci. USA* **93**:1173–1177.
57. Parekh, B. S., S. D. Sheridan, and G. W. Hatfield. 1996. Effects of integration host factor and DNA supercoiling on transcription from the *ilvP<sub>G</sub>* promoter of *Escherichia coli*. *J. Biol. Chem.* **271**:20258–20264.
58. Pérez-Martin, J., and M. Espinosa. 1991. The RepA repressor can act as a transcriptional activator by inducing DNA bends. *EMBO J.* **10**:1375–1382.
59. Pérez-Martin, J., and V. de Lorenzo. 1997. Clues and consequences of DNA bending in transcription. *Annu. Rev. Microbiol.* **51**:593–628.
60. Pruss, G. J., and K. Drlica. 1989. DNA supercoiling and prokaryotic transcription. *Cell* **56**:521–523.
61. Rao, L., W. Ross, J. A. Appleman, T. Gaal, S. Leirmo, P. J. Schlax, M. T. Record, Jr., and R. L. Gourse. 1994. Factor independent activation of *rmB* P1. An “extended” promoter with an upstream element that dramatically increases promoter strength. *J. Mol. Biol.* **235**:1421–1435.
62. Ross, W., K. K. Gosink, J. Salomon, K. Igarashi, C. Zou, A. Ishihama, K. Severinov, and R. L. Gourse. 1993. A third recognition element in bacterial promoters: DNA binding by the  $\alpha$  subunit of RNA polymerase. *Science* **262**:1407–1413.
63. Rowe-Magnus, D. A., M. Mencia, F. Rojo, M. Salas, and G. B. Spiegelman. 1998. Transcriptional activation of the *Bacillus subtilis* *spoIIG* promoter by the response regulator Spo0A is independent of the C-terminal domain of the RNA polymerase  $\alpha$  subunit. *J. Bacteriol.* **180**:4760–4763.
64. Smith, T. L., and R. T. Sauer. 1996. Dual regulation of open-complex formation and promoter clearance by Arc explains a novel repressor to activator switch. *Proc. Natl. Acad. Sci. USA* **93**:8868–8872.
65. Straney, D. C., and D. M. Crothers. 1985. Intermediates in transcription initiation from the *E. coli* *lac* UV5 promoter. *Cell* **43**:449–459.
66. Straney, D. C., S. B. Straney, and D. M. Crothers. 1989. Synergy between *Escherichia coli* CAP protein and RNA polymerase in the *lac* promoter open complex. *J. Mol. Biol.* **206**:41–57.
67. Van Sinderen, D., S. Withof, H. Boels, and G. Venema. 1990. Isolation and characterization of *comL*, a transcription unit involved in competence development in *Bacillus subtilis*. *Mol. Gen. Genet.* **224**:396–404.
68. Van Sinderen, D., A. Ten Berge, B. J. Haijema, L. Hamoen, and G. Venema. 1994. Molecular cloning and sequence of *comK*, a gene required for genetic competence in *Bacillus subtilis*. *Mol. Microbiol.* **11**:695–703.
69. Van Sinderen, D., A. Luttinger, L. Kong, D. Dubnau, G. Venema, and L. Hamoen. 1995. *comK* encodes the competence transcription factor, the key regulatory protein for competence development in *Bacillus subtilis*. *Mol. Microbiol.* **15**:455–462.
70. Venema, G., R. H. Pritchard, and T. Venema-Schröder. 1965. Fate of transforming deoxyribonucleic acid in *Bacillus subtilis*. *J. Bacteriol.* **89**:1250–1255.
71. Whipple, F. W., and A. L. Sonenshein. 1992. Mechanism of initiation of transcription by *Bacillus subtilis* RNA polymerase at several promoters. *J. Mol. Biol.* **223**:399–414.
72. Zinkel, S. S., and D. M. Crothers. 1991. Catabolite activator protein-induced DNA bending in transcription initiation. *J. Mol. Biol.* **219**:201–215.